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"Gluconeogenesis and Glycogenolysis-The 24-Hr Pattern and Correlation of Several Physiologic Functions"

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THE 24-HR PATTERN OF HEPATIC PHOSPHOHEXOSE ISOMERASE IN MICE AND RATS

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ABSTRACT: The purpose of this investigation was to determine whether or not hepatic phosphohexose isomerase (E. C. 5.3.1.9) exhibits a significant variation during a 24-hr period in rats and mice. Significant diurnal variations were found for this enzyme in both rats and mice. Synchrony in the times of the peaks and troughs, however, was not obtained in these experiments.

INTRODUCTION

D-Glucose-6-phosphate ketol-isomerase (E. C. 5.3.1.9) is also known as phosphohexose isomerase or phosphoglucose isomerase. This enzyme catalyzes the following reaction:

$$\text{D-Glucose-6-phosphate} \xrightarrow[\text{isomerase}]{\text{phosphohexose}} \text{D-fructose-6-phosphate}$$

D-Glucose-6-phosphate is converted to D-fructose-6-phosphate in the presence of phosphohexose isomerase. The reaction proceeds at the same rate in either direction (King, 1974). This enzyme is found in many animal tissues and is an enzyme of glycolysis and pentose metabolism. This enzyme has been studied in various diseases such as malignancies, genitourinary disease and the infectious disease, trypanosomiasis in guinea pigs (Marciacq, 1970). Unfortunately, temporal analyses have not been performed for this enzyme in healthy or diseased animals and it is therefore not known whether or not this enzyme exhibits a significant diurnal variation. The purpose of this investigation was to determine whether or not phosphohexose isomerase exhibits the daily fluctuations shown by many other hepatic enzymes (Ashman and Seed, submitted report).

MATERIALS AND METHODS

The animals used in this experiment were laboratory mice

obtained from the Tulane Medical School Vivarium and rats from Holtzman Company (Madison, Wisconsin).

The animals were maintained in a photoperiod room under alternating artificial light, with the lights on from 0600 to 1800 and with a temperature of 22.8° C ± 5.6° C. All animals were fed Purina Lab Chow and water ad libitum.

The mice used in Sets I and II were placed in the photoperiod room December 5, 1974. The mice were all sexually mature. The youngest mice were about three months old. Mice were killed beginning December 17, 1974. Their approximate weights at death ranged from 23 grams to 38.5 grams.

Rats used in Set I were placed in the photoperiod room August 14, 1973 and killed September 1, 1973. Group II rats were placed in the photoperiod room October 24, 1973 and killed November 16 and 17, 1973. Group III rats were placed in the photoperiod room January 15, 1974 and killed February 8 and 9, 1974. All rats were sexually mature at death. Their weights at death ranged from 246 grams to 325 grams.

All animals were killed at six time points during a 24-hr period. The livers were removed at death, weighed and stored at -70° C until analysis.

Phosphohexose isomerase was assayed by a modification of the method of Bodansky (1954). One-gram liver samples were prepared by homogenization in ground glass tissue grinders in 2.5 ml of 0.0175 M phosphate buffer, pH 7.4. The

homogenates were then centrifuged at 12,000 RPM's for 20 minutes. The supernatnat fraction was used for all enzyme analyses. The reaction was stopped by adding 2.5 ml of 5% trichloracetic acid. The deproteinized supernatant was assayed for fructose-6-phosphate using the method of Roe (1934). A Bausch and Lomb spectronic 20 was used at a wavelength of 490nm.

Statistical tests included the standard error of the means and the two-sample t-test between the means. The level of significance chosen was the 5% level. A Friden 1155 programmable calculator was used for all calculations.

RESULTS AND DISCUSSION

Figure 1 represents the diurnal variation of hepatic phosphohexose isomerase in male mice. The vertical axis is enzyme activity expressed as per cent of the highest value of umoles of fructose-6-phosphate formed per ml of protein per 15 minutes. Each point is the mean value of 3 animals killed at six time points throughout a 24-hr period. Vertical bars in brackets represent the standard error of the mean. The peak value occurred at 0600 and the trough at 0200. The difference between the peak and trough is represented by a 1.3-fold difference, significant at the 5% level. A significant diurnal variation for this enzyme was not found in the group of female mice. This does not mean that the diurnal variation does not exist in female mice.

Perhaps this particular group of females was not as well-synchronized as the male mice.

Figure 2 shows the diurnal variation of phosphohexose isomerase in male rats (Group I). The peak value occurred at 1000 hrs. and the trough at 1400 hrs. The difference between the peak and trough values was represented by a 1.2-fold difference, significant at the 2% level.

Figure 3 represents the diurnal variation of phospho-hexose isomerase in male rats (Group II). The peak value occurred at 0200 and the trough at 0600. The difference between the peak and trough values was a 1.3-fold difference, statistically significant at the 5% level.

Figure 4 illustrates the diurnal variation of phospho-hexose isomerase in a third group of rats (Group III). The peak value was at 1400 hrs and the trough at 0600 hrs. There was a 1.2-fold difference between the peak and trough and this difference was statistically significant at the 1% level.

In these experiments using nocturnal animals fed ad libitum, we found significant diurnal variations in hepatic phosphohexose isomerase in each group of animals. Synchrony in the time of the peaks and troughs, however, was not established in these experiments. This could be due to three possibilities: (1) the same portion of the liver was not used for each animal since the liver portions were destroyed during an electical shut-down in the Louisiana hurricance of

1974. We therefore had to use portions from entire livers which were saved; (2) the animals were in their experimental regimens at different times of the year; and (3) the method of assay may have to be revised according to the method of King (1974). These three possibilities will have to be considered in all future experiments. In addition, the relationship of the 24-hr pattern of this enzyme will be correlated with the 24-hr patterns of other enzymes involved in gluconeogenesis and glycogenolysis.

REFERENCES

Ashman, P. and Seed, J. R. The effects of parasitic organisms on the biorhythms of the host. Submitted to Experimental Parasitology.

Bodansky, O. 1954. Phosphohexose isomerase of serum. Cancer 7: 1191-1199.

King, John. 1974. Glucosephosphate isomerase. In: Methods of Enzymic Analysis. Vol. II, Ed. H. U. Bergmeyer, Academic Press, Inc., New York.

Marciacq, Yolanda. 1970. Biochemical changes in carbohydrate utilization during African trypanosomiasis in the guinea pig. Ph.D. Dissertation, Tulane University.

Roe, Joseph H. 1934. A colorimetric method for the determination of fructose in blood and urine. J. Biol. Chem. 107: 15-22.

Figure 1. The diurnal variation of mouse hepatic phosphohexose isomerase expressed as per cent of highest specific activity (umoles F-6-P formed/ml protein/15 min.). Each point is the mean value (± SEM). The horizontal axis is the time of day. The vertical axis is per cent of the highest specific activity. Animals were maintained under 12 hours of alternating illumination with the lights on from 0600 to 1800 hrs. The difference between the peak and trough is significant at the 5% level.

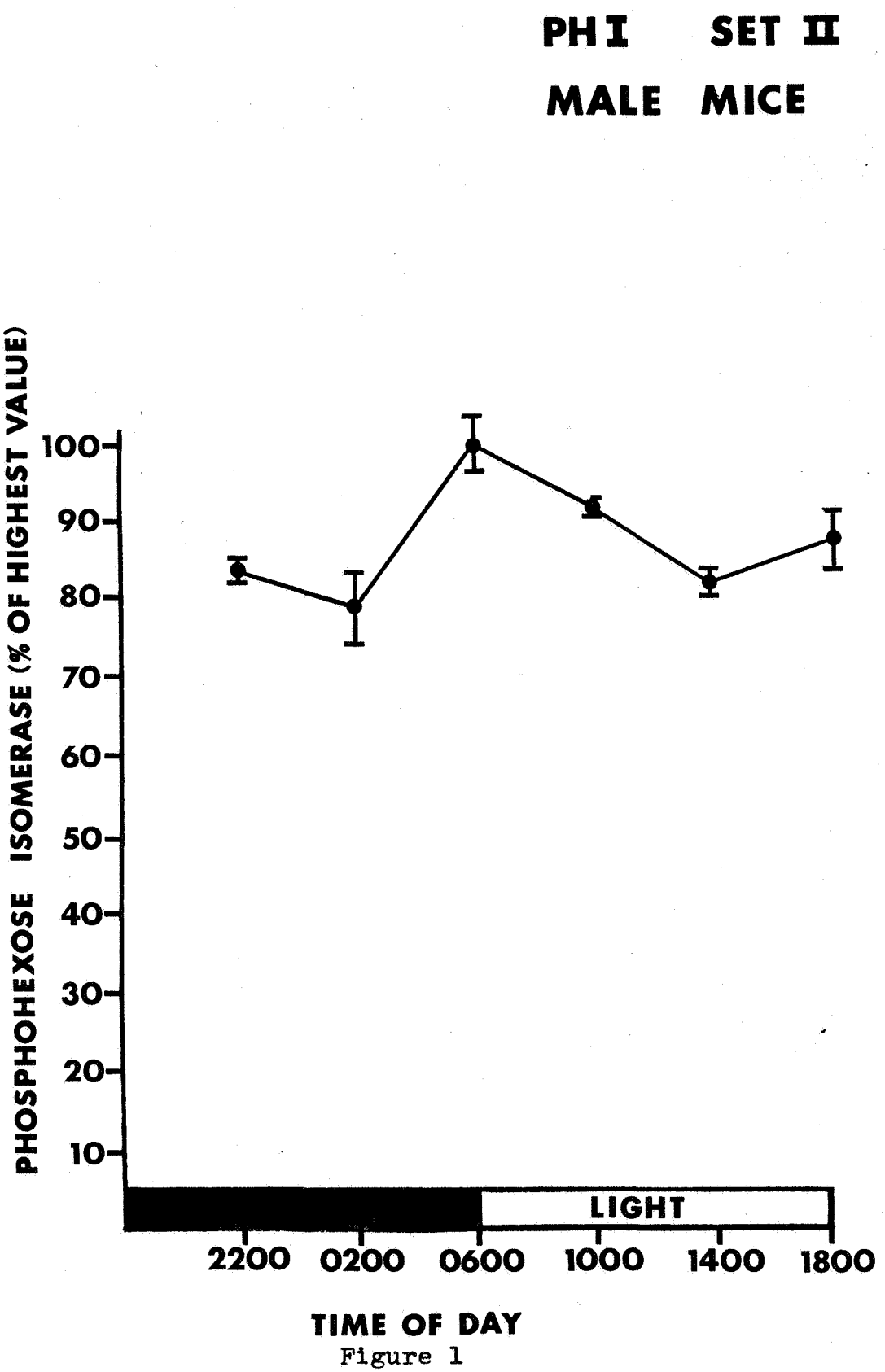
PH I SET II
MALE MICE
PHOSPHOHEXOSE ISOMERASE (% OF HIGHEST VALUE)
100
90
80
70
60
50
40
30
20
10
LIGHT
2200
0200
0600
1000
1400
1800
TIME OF DAY

Figure 1

Figures 2-4. The diurnal variation of rat phosphohexose isomerase expressed as per cent of the highest specific activity (umoles F-6-P formed/ml protein/15 min.). Animals were maintained under 12 hours of alternating illumination with lights on from 0600 to 1800 hrs. Each point is the mean value (± SEM). The vertical axis is the per cent of the highest specific activity and the horizontal axis is the time of day. In Figure 2, the difference between the peak at 1000 hrs and the trough at 1400 hrs was significant at the 2% level. In Figure 3, the peak occurred at 0200 hrs and the trough at 0600 hrs. This was significant at the 5% level. In Figure 4, the peak was at 1400 hrs and the trough at 0600 hrs. This difference was significant at the 1% level.

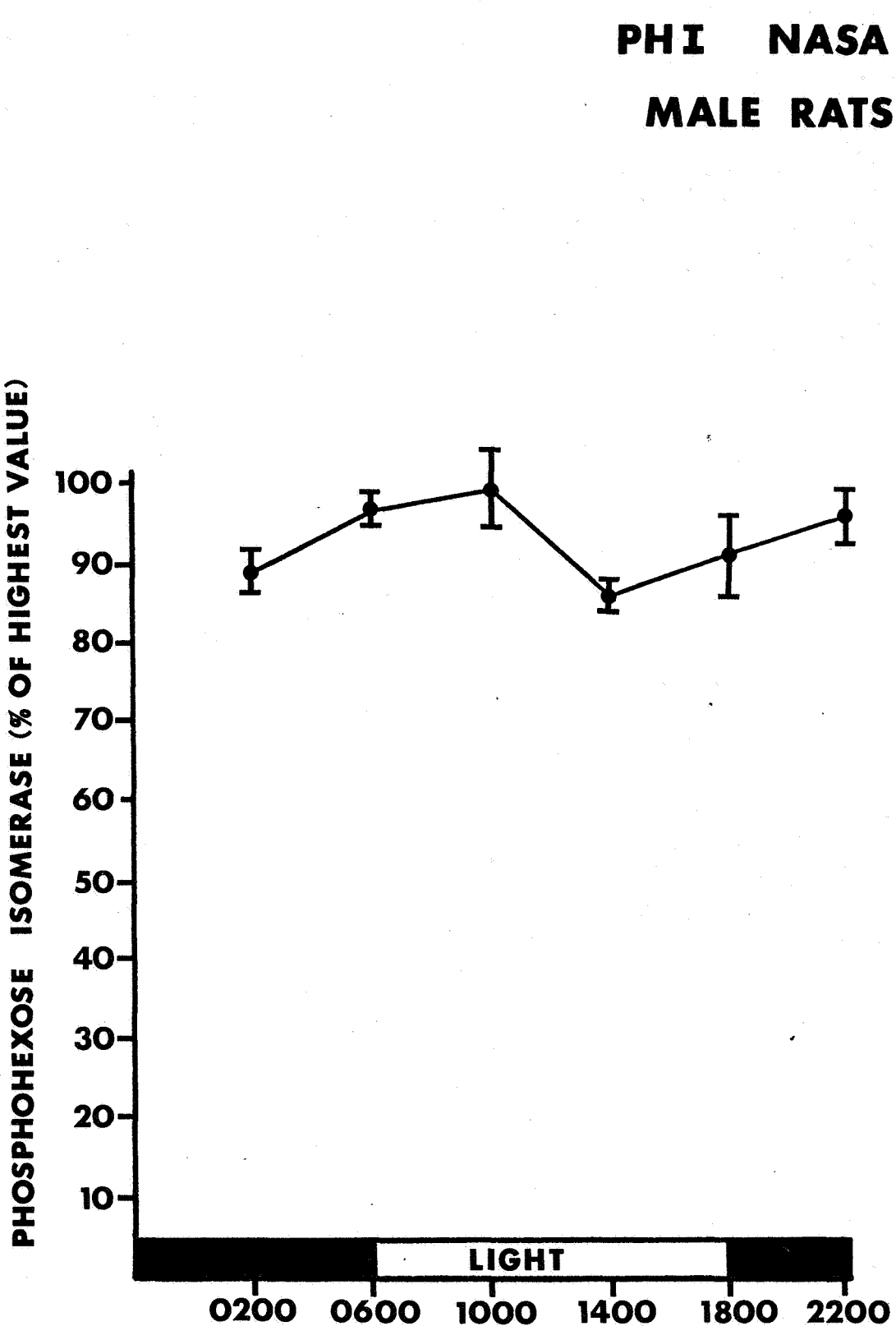
PHI NASA I
MALE RATS
PHOSPHOHEXOSE ISOMERASE (% OF HIGHEST VALUE)
100
90
80
70
60
50
40
30
20
10
LIGHT
0200
0600
1000
1400
1800
2200
TIME OF DAY

Figure 2

PHI NASA II

MALE RATS

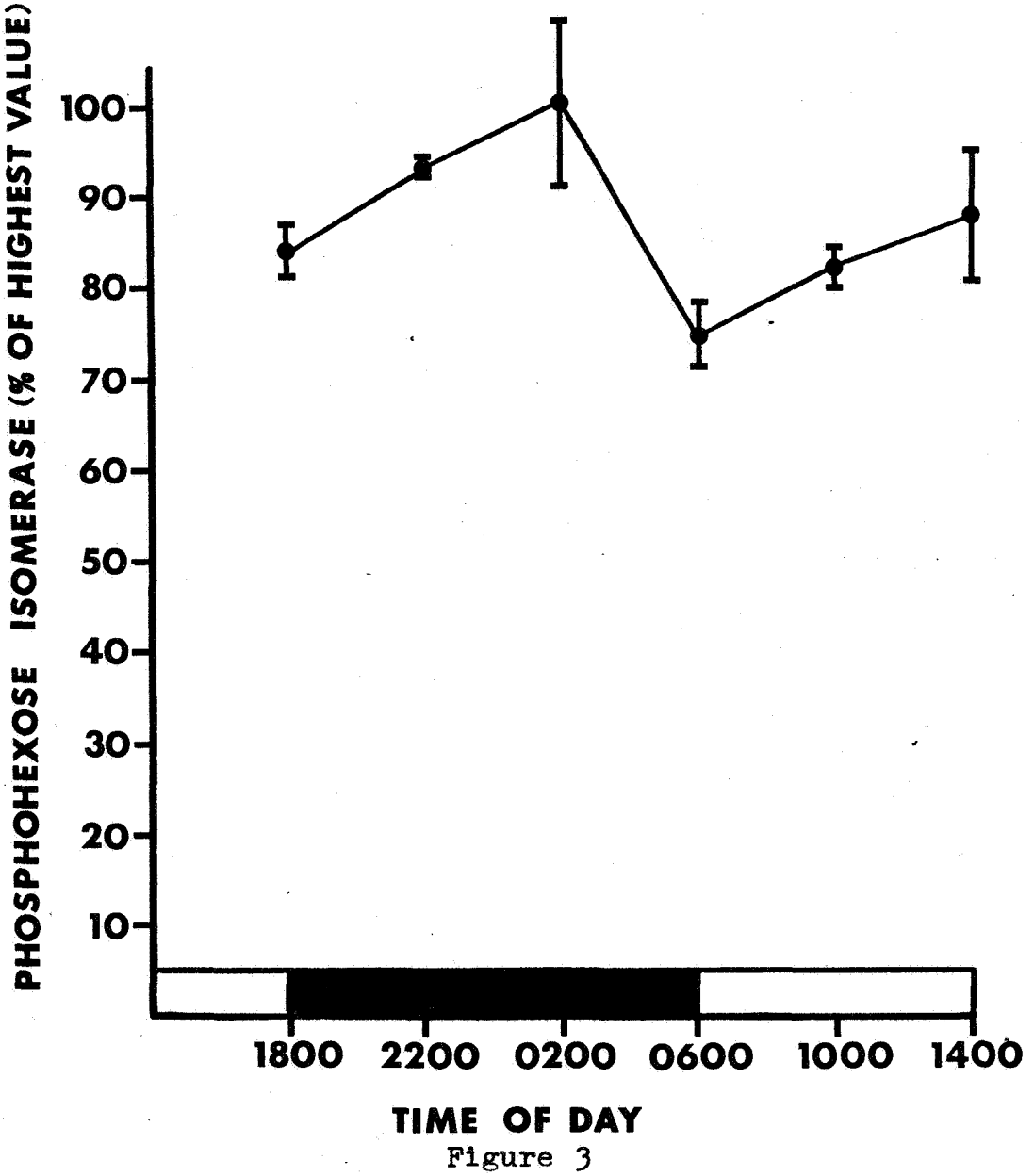

Figure 3

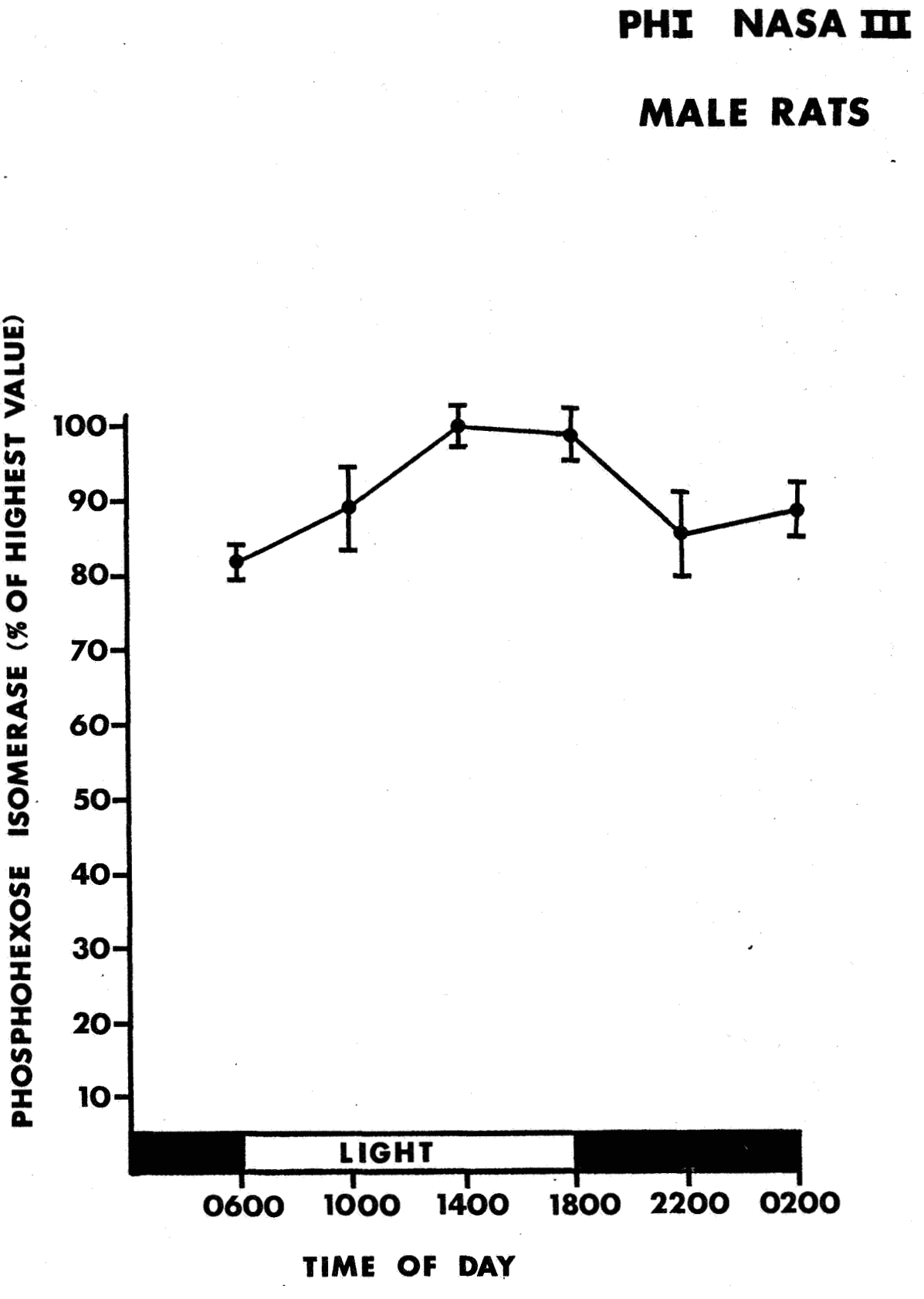
PHI NASA III
MALE RATS
PHOSPHOHEXOSE ISOMERASE (% OF HIGHEST VALUE)
100
90
80
70
60
50
40
30
20
10
LIGHT
0600
1000
1400
1800
2200
0200
TIME OF DAY

Figure 4